Eur Arch Psychiatr Neurol Sci (1985) 234:374–377

European Archives of Psychiatry and Neurological Sciences

Improvement in Neuromuscular Transmission in Myasthenia Gravis by 3,4-Diaminopyridine

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Summary. 3,4-Diaminopyridine (3,4-DAP), a potent potentiator of action potential evoked release of acetylcholine from presynaptic terminals in the neuromuscular junction was given i.v. and p.o. to two patients with myasthenia gravis. Effects were monitored electrophysiologically by repetitive nerve stimulation and by standardized clinical testing.

Administration of 8 mg and 9 mg 3,4-DAP i.v. produced a clear improvement in the neuromuscular transmission after approximately 20 min.

When 3,4-DAP was given p.o. 24 mg was shown to be effective. At a dosage of 18–24 mg p.o. 3,4-DAP significantly potentiated the effect of the cholinesterase inhibitor pyridostigmine at an optimal dose. The maximal effect of 3,4-DAP p.o. was obtained after 2.5–3 h.

No significant CNS side-effects were found which is in contrast to those reported for 4-aminopyridine.

The results suggest that 3,4-DAP may be useful as an addition to the conventional treatment with cholinesterase inhibitors when immunosuppressive treatment is considered contraindicated or when it has not yet reached its full effect.

Key words: 3,4-Diaminopyridine – Neuromuscular transmission – Myasthenia gravis

Introduction

In myasthenia gravis the critical defect is a blockade of the acetylcholine receptor at the neuromuscular junction (Grob 1979), whereas the spontaneous and evoked release of acetylcholine from the presynaptic terminals is normal or slightly enhanced (Cull-Candy et al. 1979, 1980). The beneficial effect in myasthenia gravis of treatment with anticholinesterases is well documented. The aminopyridines such as 4-aminopyridine (4-AP) and 3,4-diaminopyridine (3,4-DAP) have been shown to cause a significant increase of transmitter release in the normal neuromuscular junction (Lundh 1978; Molgo et al. 1980) and in conditions with a restricted release of acetylcholine such as botulism (Lundh et al. 1977a) and Lambert-Eaton myasthenic syndrome (LEMS), (Kim et al. 1980). Also 4-AP and 3,4-DAP have been shown clinically to ameliorate the muscle weakness in LEMS (Lundh et al. 1977b, 1983, 1984; Sanders et al. 1980). In combination with anticholinesterases, 3,4-DAP has been administered p.o. for long periods to patients with LEMS with good clinical effect and without the side-effects reported for 4-AP treatment (Lundh et al. 1984). Given i.v. to patients with myasthenia gravis 4-AP has improved the neuromuscular transmission as measured clinically and electrophysiologically (Kim et al. 1980; Lundh et al. 1979). We have now treated two patients with myasthenia gravis using 3,4-DAP i.v. and p.o. in order to find out if the drug offers further advantages in addition to the effects of an optimal anticholinesterase treatment.

Supported by the Swedish Medial Research Council, Stockholm (project no. B84-04X-00084-20C) to the Department of Clinical Neurophysiology

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Patients and Methods

Patient 1. A 68-year-old man was admitted with a 3-week history of diplopia, ptosis, masticatory and neck weakness. The diagnosis of myasthenia gravis was confirmed by an edrophonium test and by an electromyographic (EMG) decrement study. Serum acetylcholine receptor (AChR) antibody level was elevated (6.41 units/l; normal <0.2 units/l). A CT of the mediastinum was normal. A benign prostatic hypertrophy with uremia (serum creatinine 244 μmol/l; normal <115) was also found. The study was performed 1 month after hospital admittance.

Patient 2. A 62-year-old man was admitted after a 5-month history of diplopia, ptosis followed by weakness of neck flexors and proximal limb muscles. The diagnosis of myasthenia gravis was confirmed by an edrophonium test and a neurophysiological decrement study; AChR antibodies were elevated (1.8 units/l). A thymectomy was performed immediately; histological examination showed no thymic abnormalities. The study was performed 1 month after the thymectomy. The patient also had a non-insulin-dependent diabetes mellitus.

The patients were examined clinically with a test battery which was adapted individually and carefully standardized before the trial. For patient 1 the test included the number of neck flexions at about 1/s performed in a supine position, the number of chewings of a rubber spatula at about 1/s. For patient 2 the test included the time of continuous arm elevation (120°) in a standing position as well as the time of continuous elevation (60°) of each of the legs with the patient in a supine position. The clinical examination was performed once before and once during each pharmacological trial. The result of the neurophysiological decrement test served as guidance in selecting the appropriate testing time during the trial.

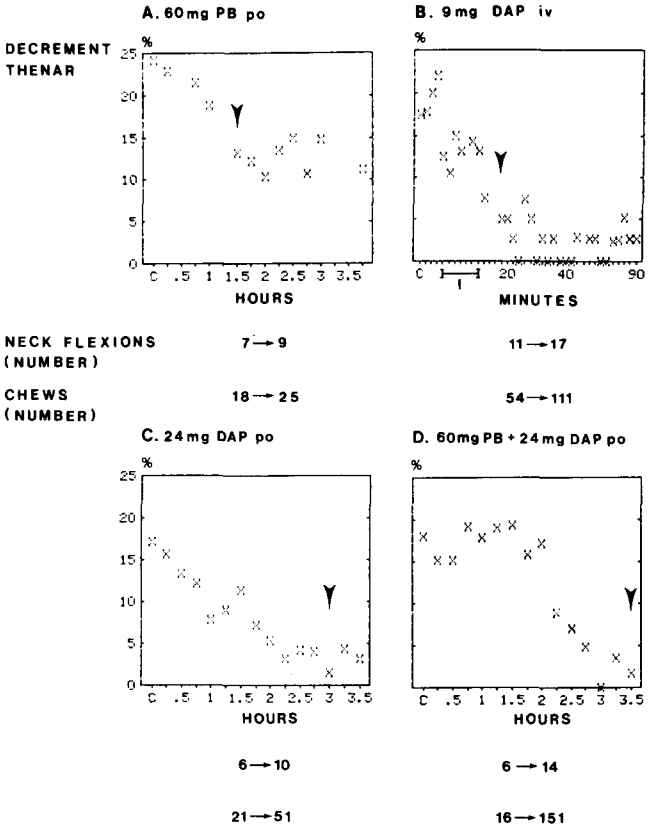

Fig. 1A–D. Effects on thenar muscle decrement (percentage decrease of thenar CMAP four stimuli at 4 Hz) of PB and 3,4-DAP at indicated dosages and routes. *Arrows* in the diagram indicate the times of the clinical testing after drug administration. Time of i.v. infusion in **B** indicated by *I*. The results of clinical testing before and after drug administration are shown below each diagram. Patient 1

Compound muscle action potentials (CMAPs) were recorded with surface electrodes from the thenar, deltoid and facial triangular muscle regions at supramaximal stimulation strengths of the respective motor nerves. The percentage decrease of CAMP amplitude at a train of four stimuli at 1, 2 and 4 Hz were determined (decrement). At the initial electrophysiological test the decrement values at 4 Hz for thenar, deltoid and facial muscles were 21%, 22% and 6% for patient 1 and 50%, 63% and 10% for patient 2, i.e., distinctly pathological. There was no significant potentiation of CMAP after maximal voluntary activation of the muscle. Nerve conduction velocities, CMAP amplitudes and sensory nerve APs were normal. Needle EMG showed no signs of denervation or myopathy.

The trial was performed after informed consent from the patients and their relatives and was approved by the Swedish Governmental Medical Authorities.

Results

At the time of the trial patient 1 was being treated with 60 mg pyridostigmine bromide (PB) three times daily producing a clinically acceptable effect. The drug was withdrawn 24 h before the test period. Premedication was given with 0.5 mg atropine i.v. before the i.v. test, and 0.4 mg hyoscyamine sulphate p.o. before the p.o. test. The tests illustrated in Fig. 1 were performed on consecutive days. Administration of 60 mg PB p.o. caused a significant decrease of thenar CMAP decrement from 1.5 h and onwards, and a moderate clinical improvement (Fig. 1A). Infusion of 9 mg 3,4-DAP (i.v. in 250 ml saline for 15 min) reduced the decrement to zero from 20 min after the end of infusion (Fig. 1B) and also caused a marked improvement in the clinical parameters. When given alone or

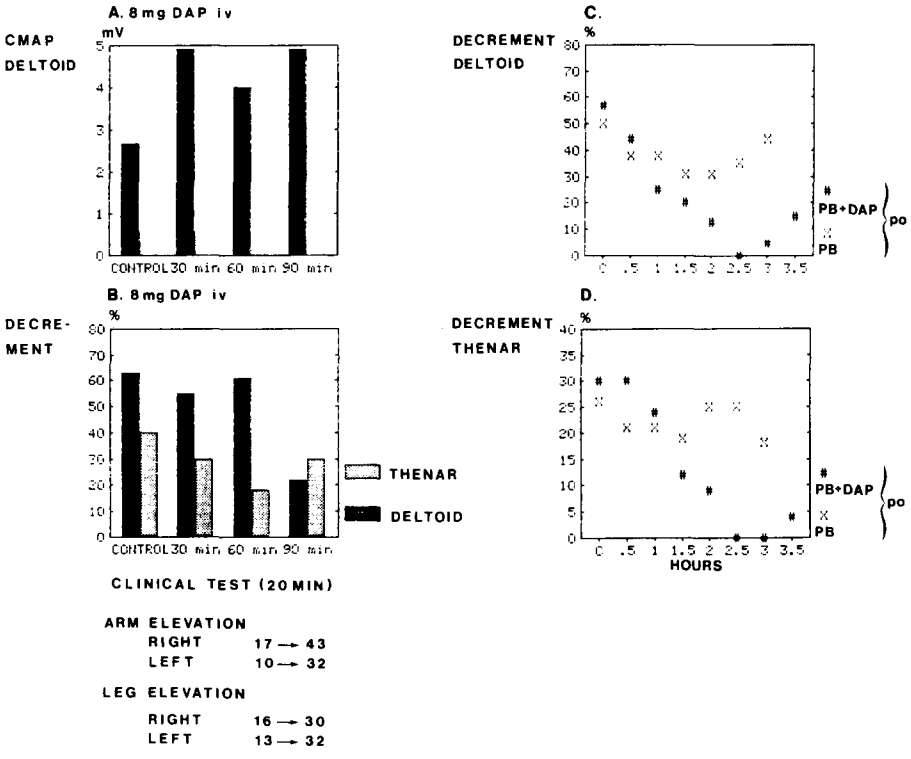

Fig. 2A–D. Time course of effect on deltoid muscle CMAP amplitude **A** and deltoid and thenar decrement **B** after i.v. administration of 3,4-DAP. Results of clinical testing given in seconds before and 20 min after drug administration at the same trial are shown below. Patient 2. **C–D** Time course of effects on deltoid and thenar muscle decrement values after p.o. administration of PB and 3,4-DAP, respectively. Patient 2

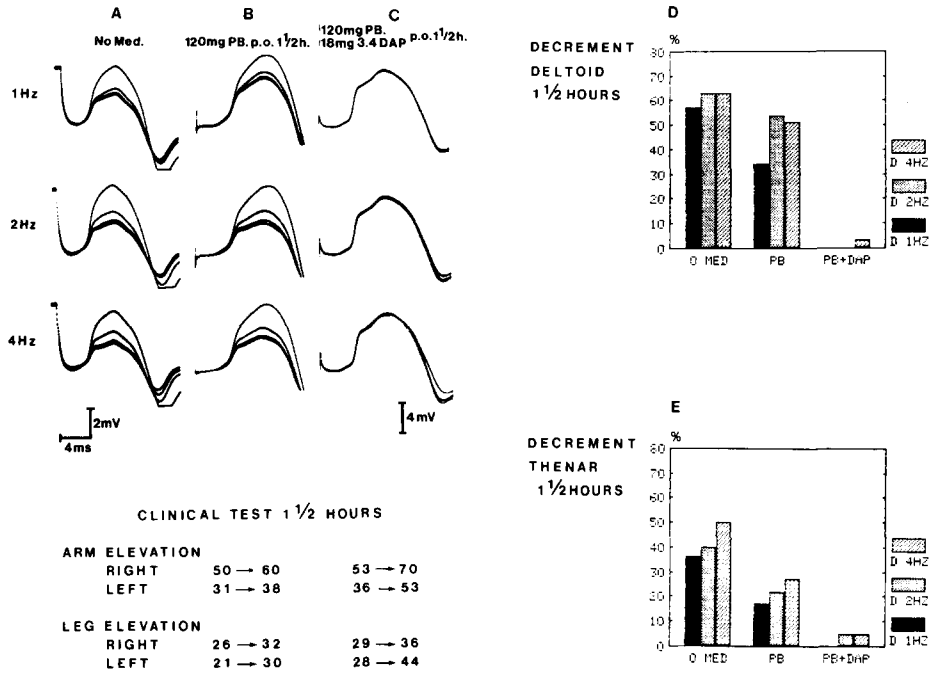

Fig. 3A–E. Result of repetitive muscle nerve stimulation before and 1.5 h after p.o. administration of PB alone and in combination with 3,4-DAP, respectively. **A–C** CMAP's recorded from the deltoid muscle at indicated frequencies of stimulation. Note different amplification in **C** versus **A**, **B**. The results of the clinical testing given in seconds before and 1.5 h after the drug intake are shown below **B** and **C**. Patient 2

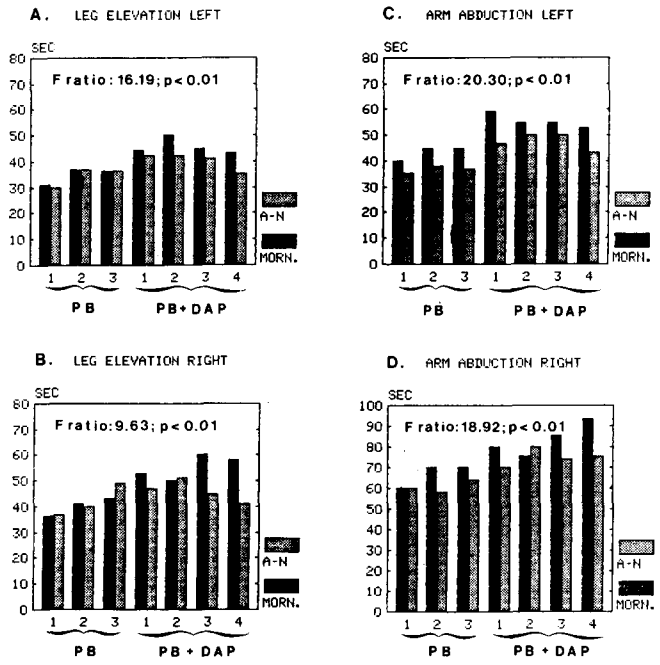

Fig. 4A–D. Results of clinical testing on consecutive days of treatment with PB alone and in combination with 3,4-DAP. For further details, see text. Patient 2

in combination with 60 mg PB, 24 mg 3,4-DAP p.o. eliminated the thenar decrement (Fig. 1C, D). However, in combination with PB the effect on the clinical parameters was much better than when given alone.

The regular medication given to patient 2 (120 mg PB four times daily) was terminated 18 h before the trial. Premedication was given with 0.5 atropine i.v. before the i.v. test, and with 0.6 mg hyoscyamine sulphate p.o. before the p.o. tests. After 8 mg 3,4-DAP in 250 ml saline given i.v. (Fig. 2A), the CMAP of the deltoid muscle showed an increase in amplitude after 30 min followed by a reduction of the decrement after 90 min (Fig. 2B). The decrement of the thenar CMAP was reduced after 60 min with simultaneous significant clinical improvement.

The effects 1.5 h after p.o. administration of 120 mg PB alone or in combination with 18 mg 3,4-DAP are shown in Fig. 3. The combined treatment totally eliminated the decremental responses in thenar and deltoid muscles whereas PB alone only produced a moderate improvement of the neuromuscular transmission. Also the clinical tests indicated a beneficial effect with 3,4-DAP and PB as compared to PB alone.

The time courses of the effects after p.o. administration are presented in Fig. 2C, D. With the combined administration of PB and 3,4-DAP a significant improvement was found after 1 h and a maximal effect was reached after 2.5 h whereas PB alone reached its maximal effects on neuromuscular transmission after 1.5–2 h.

The optimal dose of PB in patient 2 was found to be 120 mg given four times a day. Clinical testing was performed 1.5 h after the morning (7 a.m.) and afternoon (2 p.m.) doses on three consecutive days. To the morning and afternoon doses of PB 12 mg 3,4-DAP was added and clinical testing was again performed for 4 days in sequence by a person unaware of the drugs given. The results are presented in Fig. 4 together with the results of a variance analysis (ANOVA) test). The addition of 3,4-DAP to the PB medication significantly increased the muscle power of proximal limb muscles with a tendency of increasing the difference between the morning and afternoon values (F ratio 5,36; $P < 0.05$).

Adverse effects

Both patients reported transient perioral paresthesia lasting for 15 to 30 min after 3,4-DAP given as a single i.v. (9 and 8 mg) or p.o. (24 and 18 mg) dose. After the i.v. infusion both patients reported a bursting sensation in the infused arm for about 1.5 h. These side-effects did not concern the patients in any significant way. Patient 2 reported no side-effects after 5 days of continuous medication with 12 mg, 3,4-DAP twice a day.

Discussion

Having demonstrated that i.v. and p.o. administration of 3,4-DAP improves the neuromuscular transmission in myasthenia gravis as compared with anticholinesterase treatment alone, the next question is: is there a place for aminopyridine treatment in myasthenia gravis? Clinical situations where reinforced symptomatic treatment is desirable are (a) the critical period while waiting for a remission after the initiation of immunosuppressive treatment, (b) during the period of clinical deterioration sometimes preceding the effect of immunosuppressive treatment (Matell 1967), (c) when such treatment does not result in acceptable long-term benefit, and (d) sometimes immunosuppressive treatment is considered contraindicated.

Our trial in patient 2 shows that the clinical effect of 3,4-DAP treatment was maintained for 5 days of continuous treatment. One possible difficulty with long-term treatment using aminopyridines may be development of tolerance to the drug, possibly due to depletion of transmitter from the presynaptic terminals. By keeping the daily dose of 3,4-DAP as low as 24 mg development of tolerance has been avoided in a case of LEMS who has now been successfully treated for 21 months (Lundh et al. 1984).

Combination of two therapeutic principles, i.e., cholinesterase inhibition and potentiation of transmitter release makes it possible to attain significant therapeutic effects on neuromuscular transmission in conditions of defective transmitter release such as LEMS (Lundh et al. 1979) or in conditions with blockade of acetylcholine receptor by tubocurarine (Miller et al. 1979) or by immunological mechanisms such as in myasthenia gravis. The explanation for the significant effect of the aminopyridines in myasthenia gravis in addition to that produced by anticholinesterase inhibition is that these drugs increase the normal content of acetylcholine at presynaptic discharge frequencies below 50 Hz (Lundh 1978), which is well within the physiological range of motor unit firing Hannerz 1974).

In lacking acute side-effects such as anxiety, ataxia, and epileptic fits at therapeutic levels 3,4-DAP offers significant advantages over 4-AP, probably due to its higher potency on neuromuscular transmission (Molgo et al. 1980) and its weaker effects on CNS (Lechat et al. 1968). Side-effects due to cholinergic overstimulation would be expected to be potentiated by 3,4-DAP as well as 4-AP and a combination with an anticholinergic drug is recommended at the clinical trial. The long-term toxic effects of the 3,4-DAP have not been systematically investigated. Our two patients with LEMS have now been treated for 21 and 15 months and have shown no signs of toxic effects despite an intensive follow-up.

We consider the first experiences of adding 3,4-DAP to the therapeutic regime in myasthenia gravis promising enough to pursure the trials. Because the group of myasthenia gravis patients in which routine treatment has insufficient effect constitutes a much larger population of patients than those with LEMS, we find it imperative that a proper toxicity investigation of the drug is performed.

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Received March 15, 1985